

## For immunotherapy discovery

# Ready-to-Use CAR-T Cells and Target Cell Lines

AMS BIO's CAR-T new product development programs are being designed for pre-clinical and future clinical applications. CAR-T cells can be used for:

1. Compound screening
2. Antibody screening
3. Co-stimulatory and activation domain comparison
4. Personalized medicine and donor variations for CAR-T screening
5. Checkpoint inhibitors
6. Safety switches and regulators of CAR-T functions
7. Pre-clinical *in vivo* models
8. Treg and T memory cells in CAR-T setting
9. CAR-T signaling, tumor microenvironment
10. Proof of concept studies for clinical trials

### The structure of CAR from AMS BIO's available CAR-T cells targeting CD19 with inducible caspase-9

The inducible caspase-9-CD19 CAR construct consists of FKBP12 gene with mutation F36 to V coding two 12 kDa FK506 binding proteins fused to truncated Caspase9 (135-416; lacking pro-domain), followed by T2A peptide and then CD19scFv-CD28-CD3zeta. Administration of chemical inductor of dimerization (CID) CID (AP1903 or AP20187) results in dimerization of caspase 9 leading to an activated form of the molecule, which then initiates an apoptotic signaling leading to apoptosis of the transduced cell.

Introduction of inducible caspase-9 is used for increasing safety of CAR-T cells by decreasing cytotoxicity of CAR-T cells in the presence of dimerization agent.

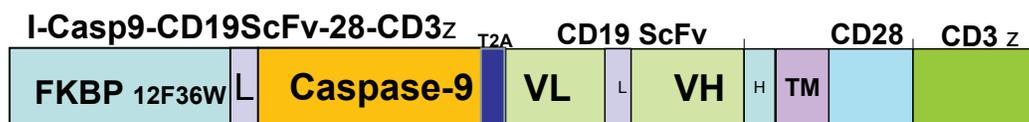


Figure 1. CAR-T cells expressing the above construct are available from AMS BIO targeting CD19 with inducible caspase-9. Both genes are separated with T2A peptide sequence allowing independent expression of both gene products. ScFv, single chain variable fragment.

To date AMSBIO generated 2nd or 3rd generation CAR and CAR controls (2nd generation of CAR is shown in Figure 1), CAR-T cells and CAR-Natural Killer (NK) effector cells against cancer target cells that show excellent functionality, including dose-dependent and target cell-specific cytotoxic activity (Figure 2,3).

These cells can be tested with CAR-T in cytotoxic assays and used for testing modulators of immune checkpoint inhibitors (PD-1, CTLA-4 pathways) or activators of immune response, small molecules affecting T cell or Treg activity.

### icasp9-CD19 CAR-T cells

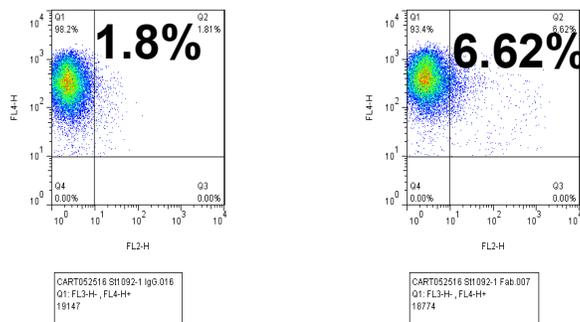


Figure 2. Flow cytometry with Fab Ab showing expression of scFv.

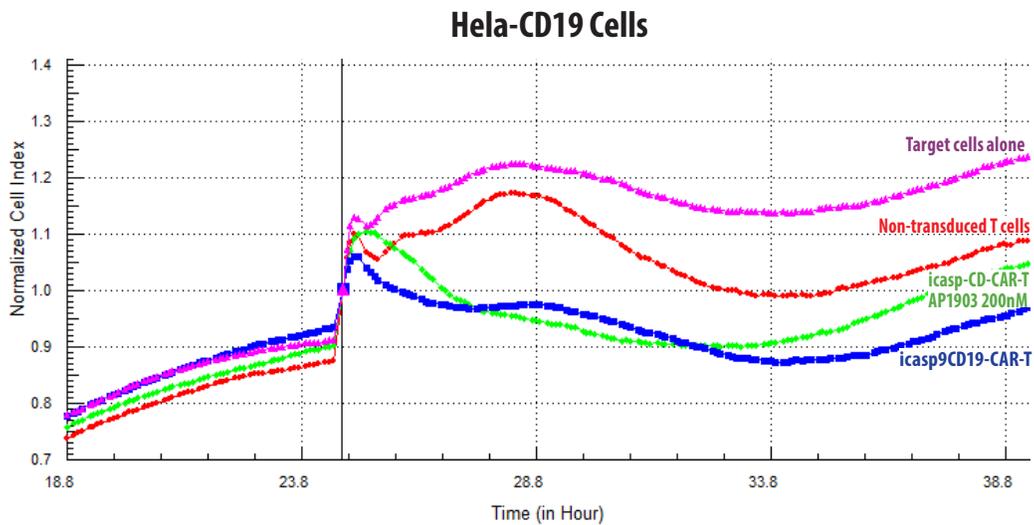


Figure 3. RTCA assay shows decrease of cytotoxicity of icasp9CD19 CAR-T cells in the presence of chemical inductor dimerization (CID) AP1903 (200 nm).